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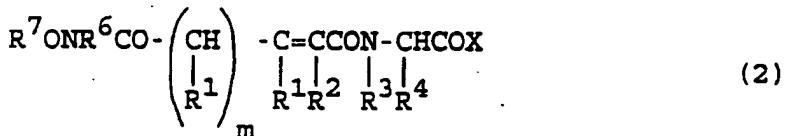
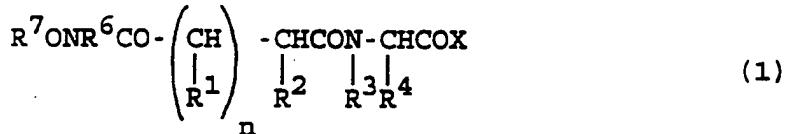
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(54) Title: IMPROVED MATRIX METALLOPROTEASE INHIBITORS



(57) Abstract

Compounds of formulas (1) or (2) wherein each R¹ is independently H or alkyl (1-8C) and R² is alkyl (1-8C) or wherein the proximal R¹ and R² taken together are -(CH₂)_p- wherein p = 3-5; R³ is H or alkyl (1-4C); R⁴ is fused or conjugated unsubstituted or substituted bicycloaryl methylene; n is 0, 1 or 2; m is 0 or 1; and X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), arylalkyl (6-16C); or X is an amino acid residue or amide thereof; or X is the residue of a cyclic amine or heterocyclic amine; wherein R⁶ is H or lower alkyl (1-4C) and R⁷ is H, lower alkyl (1-4C) or an acyl group, and wherein -CONR³- is optionally in modified isosteric form are useful for treating conditions which are characterized by unwanted matrix metalloprotease activities.

* See back of page

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IMPROVED MATRIX METALLOPROTEASE INHIBITORS

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Technical Field

The invention is directed to pharmaceuticals which are useful in diseases characterized by unwanted collagenase activity. More specifically, the invention concerns substituted or unsubstituted hydroxamates which include fused or conjugated bicycloaryl substituents.

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Background Art

There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metallo-protease enzymes, as are the angiotensin-converting enzymes and the enkephalinases.

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Collagenase and related enzymes are important in mediating the symptomology of a number of diseases, including rheumatoid arthritis (Mullins, D.E., et al., Biochim Biophys Acta (1983) 695:117-214); the metastasis of tumor cells (ibid., Broadhurst, M.J., et al., EP application 276436 (1987), Reich, R., et al., Cancer Res (1988) 48:3307-3312); and various ulcerated conditions. Ulcerative conditions can result in the cornea as the

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result of alkali burns or as a result of infection by Pseudomonas aeruginosa, Acanthamoeba, Herpes simplex and vaccinia viruses. Other conditions characterized by unwanted matrix metalloprotease activity include periodontal disease, epidermolysis bullosa and scleritis.

In view of the involvement of collagenase in a number of disease conditions, attempts have been made to prepare inhibitors to this enzyme. A number of such inhibitors are disclosed in EP applications 126,974 (published 1984) and 159,396 (published 1985) assigned to G.D. Searle. These inhibitors are secondary amines which contain oxo substituents at the 2-position in both substituents bonded to the amino nitrogen.

More closely related to the compounds of the present invention are those disclosed in U.S. patents 4,599,361 and 4,743,587, also assigned to G.D. Searle. These compounds are hydroxylamine dipeptide derivatives which contain, as a part of the compound, a tyrosine or derivatized tyrosine residue or certain analogs thereof.

Other compounds that contain sulphhydryl moieties as well as residues of aromatic amino acids such as phenylalanine and tryptophan are disclosed in PCT application WO88/06890. Some of these compounds also contain i-butyl side chains.

Inhibitors have also been disclosed for the related protease, thermolysin. These include hydroxamic peptide derivatives described by Nishino, N., et al., Biochemistry (1979) 18:4340-4347; Nishino, N., et al., Biochemistry (1978) 17:2846-2850. Tryptophan is also known to be therapeutic in various conditions, some of which may involve collagenase (see, for example, JP 57/058626; U.S. 4,698,342; 4,291,048). Also, inhibitors of bacterial collagenases have been disclosed in U.S. 4,558,034.

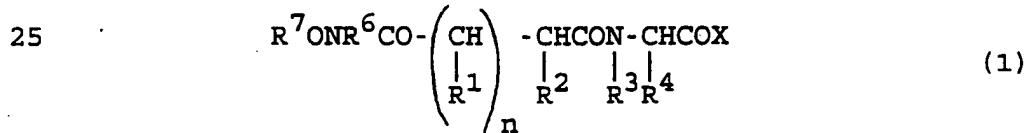
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It has now been found that the compounds described below have superior inhibiting activity with respect to matrix metalloproteases. The invention compounds add to the repertoire of agents available for 5 the treatment of conditions and diseases which are characterized by unwanted activity by the class of proteins which destroy structural proteins and designated "matrix metalloprotease" herein.

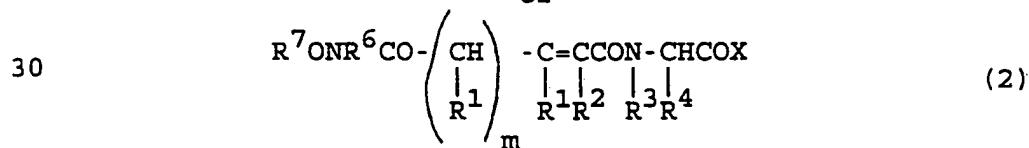
10 Disclosure of the Invention

The invention provides new compounds which are useful as inhibitors of matrix metalloproteases and which are effective in treating conditions characterized by excess activity of these enzymes. In addition, these 15 compounds can be used to purify matrix metalloproteases and to detect increased levels of matrix metalloproteases *in vivo*. The compounds take advantage of the incorporation of tryptophan or other fused or conjugated bicycloaromatic amino acid residues into a substituted or 20 unsubstituted hydroxamate-derivatized matrix metalloprotease inhibitor.

Accordingly, in one aspect, the invention is directed to compounds of the formula:



or



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wherein each R¹ is independently H or alkyl (1-8C) and R² is alkyl (1-8C) or wherein the proximal R¹ and R² taken together are -(CH₂)_p- wherein p = 3-5;

R³ is H or alkyl (1-4C);

5 R⁴ is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

10 X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or heterocyclic amine; and

15 R⁶ is H or lower alkyl (1-4C) and R⁷ is H, lower alkyl (1-4C) or an acyl group.

Also included within the scope of the invention are compounds wherein the -CONR³- amide bond shown is replaced by a modified isosteric bond, such as -CH₂NR³-,
20 -CH₂CHR³- , -CH=CR³- , -COCHR³- , -CHOHCHR³- , -NR³CO- , -CF=CR³- , and the like.

These compounds have the ability to inhibit at least one mammalian matrix metalloprotease. Accordingly, in other aspects, the invention is directed to pharmaceutical compositions containing the compounds of formula 1 or 2, to methods of treating diseases characterized by matrix metalloprotease activity using these compounds or the pharmaceutical compositions thereof. Matrix metalloproteases at a particularly undesired location can be targeted by conjugating the compounds of the invention to a targeting ligand specific for a marker at that location such as an antibody or fragment thereof or a receptor ligand.

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The invention is also directed to various other processes which take advantage of the unique properties of these compounds. Thus, in another aspect, the invention is directed to the compounds of formulas 1 or 2 conjugated to solid supports. These conjugates can be used as affinity reagents for the purification of a desired matrix metalloprotease.

In another aspect, the invention is directed to the compounds of formula 1 or 2 conjugated to label. As the compounds of the invention selectively bind to at least one matrix metalloprotease, the label can be used to detect the presence of unusually large amounts of matrix metalloprotease in vivo or in vitro cell culture.

In addition, the compounds of formula 1 or 2 can be conjugated to carriers which permit the use of these compounds in immunization protocols to prepare antibodies specifically immunoreactive with the compounds of the invention. These antibodies are then useful both in therapy and in monitoring the dosage of the inhibitors.

In still another aspect, the invention is directed to methods to prepare the compounds of formulas 1 and 2 and to novel intermediates in their preparation.

25 Brief Description of the Drawings

Figure 1 is a graph which shows the effect of the inhibitor of the invention on corneal burns using a clinical scoring method.

30 Figure 2 shows the corresponding effect of the compound of the invention on corneal burns using a perforation criterion.

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Modes of Carrying Out the Invention

The invention compounds are inhibitors of mammalian matrix metalloproteases. As used herein, "mammalian matrix metalloprotease" means any enzyme found in mammalian sources which is capable of catalyzing the breakdown of collagen, gelatin or proteoglycan under suitable assay conditions. Appropriate assay conditions can be found, for example, in U.S. patent 4,743,587, which references the procedure of Cawston, et al., Anal Biochem (1979) 99:340-345, use of a synthetic substrate is described by Weingarten, H., et al., Biochem Biophys Res Comm (1984) 139:1184-1187. Any standard method for analyzing the breakdown of these structural proteins can, of course, be used. The matrix metalloprotease enzymes referred to in the herein invention are all zinc-containing proteases which are similar in structure to, for example, human stromelysin or skin fibroblast collagenase.

The ability of candidate compounds to inhibit matrix metalloprotease activity can, of course, be tested in the assays described above. Isolated matrix metalloprotease enzymes can be used to confirm the inhibiting activity of the invention compounds, or crude extracts which contain the range of enzymes capable of tissue breakdown can be used.

The invention compounds can be considered to comprise two components linked by an amide or modified amide bond. One component is substituted or unsubstituted hydroxamate derivative of a dicarboxylic acid backbone, wherein the alternate carboxyl group forms the amide or modified bond with an amino acid or analog thereof. The amino acid or analog is the residue of an amino acid or analog which contains a fused or conjugated bicycloaromatic system, such as a tryptophan residue or a

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naphthylalanyl residue. This residue can also be amidated or can be extended by one or two additional amino acid residues. Thus, the compounds of the invention can be prepared by reaction of the corresponding underivatized compounds which are carboxylic acids or esters of the dicarboxylic residue with suitable reagents.

The dicarboxylic acid residue in formula 1 contains at least one and, in some instances, two or more chiral centers. Either configuration at any chiral center is included within the invention, as are mixtures of compounds containing the two possible configurations at each point of chirality. However, it is generally found that a particular configuration at each of these chiral centers is preferred. Similarly, in the compounds of formula 2, the double bond can be either the cis or trans configuration. In this case, also, one or the other configuration for a particular set of embodiments will be preferred. The carbon to which R⁴ is bound is chiral in both formulas. While both configurations are included in the invention, that corresponding to an L-amino acid is preferred.

The amide bond shown as -CONR³- in the compounds of formulas 1 and 2 may be in "modified isosteric" form. Compounds of the invention where this is the case are preferred when oral administration is desirable. By "modified isosteric form" is meant that in lieu of the functionality -CONR³-, the compound has instead a moiety such as those selected from the group consisting of -CH₂NR³-, -CH-NR³, -COCHR³-, -CH(OH)NR³, -CH(OH)CHR³-, -CSCHR³-, -CH=CR³-, CF=CR³ and -NR³CO-.

As used herein, "alkyl" has its conventional meaning as a straight chain, branched chain or cyclic saturated hydrocarbyl residue such as methyl, ethyl,

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isobutyl, cyclohexyl, t-butyl or the like. The alkyl substituents of the invention are of the number of carbons noted which may be substituted with 1 or 2 substituents. Substituents are generally those which do not interfere with the activity of the compound, including hydroxyl, "CBZ," amino, and the like. Aryl refers to aromatic ring systems such as phenyl, naphthyl, pyridyl, quinolyl, indolyl, and the like; aryl alkyl refers to aryl residues linked to the position indicated through an alkyl residue. In all cases the aryl portion may be substituted or unsubstituted. "Acyl" refers to a substituent of the formula RCO- wherein R is alkyl or arylalkyl as above-defined. The number of carbons in the acyl group is generally 1-15; however as the acyl substitute is readily hydroxylized *in vivo* the nature of the group is relatively unimportant. "Cyclic amines" refer to those amines where the nitrogen is part of a heterocyclic ring, such as piperidine, "heterocyclic amines" refer to such heterocycles which contain an additional heteroatom, such as morpholine.

In the compounds of formula 1, preferred embodiments for R^1 and R^2 include those wherein each R^1 is H or Me and R^2 is alkyl of 3-8C, especially isobutyl, 2-methyl butyl, or isopropyl. Especially preferred is isobutyl. Preferred also are those compounds of formula 1 or 2 wherein $n=1$ or $m=1$.

In both formula 1 and 2 compounds, preferred embodiments of R^3 are H and methyl, especially H.

R^4 is a fused or conjugated bicyclo aromatic system linked through a methylene group to the molecule. By "fused or conjugated bicyclo aromatic system" is meant a two-ringed system with aromatic character which may, further, contain one or more heteroatoms such as S, N, or O. When a heteroatom such as N is included, the system

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as it forms a part of formula (1) or (2), may contain an acyl protecting group (1-5C) attached to the nitrogen.

Representative bicyclo fused aromatic systems include naphthyl, indolyl, quinolinyl, and isoquinolinyl.

- 5 Representative conjugated systems include biphenyl, 4-phenylpyrimidyl, 3-phenylpyridyl and the like. In all cases, any available position of the fused or conjugated bicyclic system can be used for attachment through the methylene. The fused or conjugated aromatic system may
10 further be substituted by 1-2 alkyl (1-4C) residues and/or hydroxy or any ring nitrogens may be acylated.
Preferred acylation is acetylation.

- Preferred embodiments of R⁴ include 1-(2-methyl naphthyl)methylene; 1-quinolyl methylene; 1-naphthyl
15 methylene; 2-naphthyl methylene; 1-isoquinolyl methylene; 3-isoquinolyl methylene; 3-thionaphthetyl methylene; 3-cumaronyl methylene; 3-(5-methylindolyl)methylene; 3-(5-hydroxyindolyl)methylene; 3-(2-hydroxyindolyl)-methylene; biphenyl methylene; and 4-phenylpyrimidyl
20 methylene; and the substituted forms thereof.

Many of these substituents as part of an amino acid residue are described in Greenstein and Winitz, "Chemistry of the Amino Acids" (1961) 3:2731-2741 (John Wiley & Sons, NY).

- 25 A particularly preferred embodiment of R⁴ is 3-indolyl methylene or its N-acylated derivative--i.e., that embodiment wherein the "C-terminal" amino acid is a tryptophan residue or a protected form thereof. A preferred configuration at the carbon to which R⁴ is
30 bound is that corresponding to L-tryptophan.

- Preferred embodiments of X are those of the formula NHR⁵ where R⁵ is H, substituted or unsubstituted alkyl (1-12C) or aryl alkyl (6-12C). Particularly preferred substitutions on R⁵ are a hydroxyl group, or a
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phenylmethoxycarbamyl (CBZ) residue. In addition, the compound may be extended by embodiments wherein X is an additional amino acid residue, particularly a glycyl residue, which may also be amidated as described.

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Therapeutic Use of the Compounds of the Invention

As set forth in the Background section above, a number of diseases are known to be mediated by excess or undesired matrix-destroying metalloprotease activity.

10 These include tumor metastasis, rheumatoid arthritis, skin inflammation, ulcerations, particularly of the cornea, reaction to infection, and the like. Thus, the compounds of the invention are useful in therapy with regard to conditions involving this unwanted activity.

15 The invention compounds can therefore be formulated into pharmaceutical compositions for use in treatment or prophylaxis of these conditions. Standard pharmaceutical formulation techniques are used, such as those disclosed in Remington's Pharmaceutical Sciences,
20 Mack Publishing Company, Easton, PA, latest edition.

For indications to be treated systemically, it is preferred that the compounds be injected. These conditions include rheumatoid arthritis and tumor metastasis. The compounds can be formulated for
25 injection using excipients conventional for such purpose such as physiological saline, Hank's solution, Ringer's solution, and the like. Injection can be intravenous, intramuscular, intraperitoneal or subcutaneous. Dosage levels are of the order of 0.1 µg/kg of subject to
30 1 mg/kg of subject, depending, of course, on the nature of the condition, the nature of the subject, the particular embodiment of the invention compounds chosen, and the nature of the formulation and route of administration.

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In addition to administration by injection, the compounds of the invention can also be formulated into compositions for transdermal or transmucosal delivery by including agents which effect penetration of these tissues, such as bile salts, fusidic acid derivatives, cholic acid, and the like. The invention compounds can also be used in liposome-based delivery systems and in formulations for topical and oral administration depending on the nature of the condition to be treated.

Oral administration is especially advantageous for those compounds wherein the moiety -CONR³- is in a modified isosteric form. These compounds resist the hydrolytic action of the digestive tract. Oral formulations include syrups, tablets, capsules, and the like, or the compound may be administered in food or juice.

The inhibitors of the invention can be targeted to specific locations where the matrix metalloprotease is accumulated by using targeting ligands. For example, to focus the inhibitors to matrix metalloprotease contained in a tumor, the inhibitor is conjugated to an antibody or fragment thereof which is immunoreactive with a tumor marker as is generally understood in the preparation of immunotoxins in general. The targeting ligand can also be a ligand suitable for a receptor which is present on the tumor. Any targeting ligand which specifically reacts with a marker for the intended target tissue can be used. Methods for coupling the invention compound to the targeting ligand are well known and are similar to those described below for coupling to carrier. The conjugates are formulated and administered as described above.

For localized conditions, topical administration is preferred. For example, to treat ulcerated cornea, direct application to the affected eye may employ

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a formulation as eyedrops or aerosol. For corneal treatment, the compounds of the invention can also be formulated as gels or ointments, or can be incorporated into collagen or a hydrophilic polymer shield. The 5 materials can also be inserted as a contact lens or reservoir or as a subconjunctival formulation. For treatment of skin inflammation, the compound is applied locally and topically, in a gel, paste, salve or ointment. The mode of treatment thus reflects the nature 10 of the condition and suitable formulations for any selected route are available in the art.

Particular topical conditions that are susceptible to treatment using the compounds of the invention include stomach ulcers, superficial wounds of any type, 15 epidermolysis bullosa, various forms of skin cancer, and pemphigus. It is well known that collagenase is involved in the progress of stomach ulcers, as described by Hasabe, T., et al., J Exp Clin Med (1987) 12:181-190; Ozaki, I., et al., Scand J Gastroenterol Suppl (Norway) 20 (1989) 16:138-141. Collagenase is also found in marginal wound tissue as reviewed by Mullins, D.E., et al., Biochim Biophys Acta (1983) 695:177-214, and patients showing impaired wound healing are known to have increased collagenase activity in the wound tissue. For 25 example, Hennesey, P.J., et al., J Pediat Surg (1990) 25:75-78, showed this to be the case in diabetes; Sank, A., et al., Surgery (1989) 106:1141-1148, demonstrated this enhanced collagenase activity in paraplegia, chronic renal failure, and Cushings disease. Thus, the 30 collagenase inhibitors of the invention are useful in any ulcerative skin condition, including, for example, decubitus ulcers, or other conditions where wound healing is slow, other conditions susceptible to treatment by the compounds of the invention include corneal or scleral

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melting associated with keratomalacia, scleromalacia perforans and connective tissue diseases.

This is also the case with respect to wounds inflicted in the course of medical treatment. It has
5 been demonstrated that collagenase inhibitors enhance the strength of suture lines and suture holding capacity in intestinal anastomoses in rats (Hogstrom, H., et al., Res Exp Med (1985) 185:451-455. Other topical conditions responsive to the collagenase inhibitors of the invention include epidermolysis bullosa where increased collagenase activity has been demonstrated (Perez-Tamayo, R., Am J Path (1978) 92:509-566, pp. 539-541). It is also known that collagenase activity is increased in the stroma surrounding basal cell carcinoma and throughout tumor
10 stromas in ulcerated basal cell carcinoma (Childer, S.,
15 J.W., et al., J Am Acad Dermatol (1987) 17:1025-1032).

Further, the matrix metalloprotease inhibitors of the invention are useful in the treatment of septic shock and in the treatment of adult respiratory distress syndrome (ARDS). The role of plasma proteases in septic shock has been shown by Colman, R.C., New Eng J Med (1989) 320:1207-1210, and the participation of neutrophil proteases in both septic shock and ARDS has been shown by Idell, S., et al., Ann Res Respir Dis (1985) 132:1098-
20 1105. In the case of these conditions, the compounds of the invention protect the connective tissue directly from digestion by matrix metalloproteases as well as prevent the degradation of collagen and therefore prevention of known chemotaxis of neutrophils toward collagen fragments
25 (Werb, Z., in "Textbook of Rheumatology," Kelley, W.N., et al., eds. (1989) W.B. Saunders, Philadelphia, 3rd ed., pp. 300-320). The compounds of the invention also prevent the inactivation of plasma protease inhibitors by matrix metalloproteases (Werb, Z. supra).

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In all of the foregoing, of course, the compounds of the invention can be administered alone or as mixtures, and the compositions may further include additional drugs or excipients as appropriate for the
5 indication.

Some of the compounds of the invention also inhibit bacterial metalloproteases although generally at a lower level than that exhibited with respect to mammalian metalloproteases. Some bacterial
10 metalloproteases seem to be less dependent on the stereochemistry of the inhibitor, whereas substantial differences are found between diastereomers in their ability to inactivate the mammalian proteases. Thus,
15 this pattern of activity can be used to distinguish between the mammalian and bacterial enzymes.

Preparation and Use of Antibodies

The invention compounds can also be utilized in immunization protocols to obtain antisera immunospecific for the invention compounds. As the invention compounds are relatively small haptens, they are advantageously coupled to antigenically neutral carriers such as the conventionally used keyhole limpet hemocyanin (KLH) or serum albumin carriers. Coupling to carrier can be done
20 by methods generally known in the art; the -COX functionality of the invention compounds offers a particularly convenient site for application of these techniques. For example, the COX residue can be reduced
25 to an aldehyde and coupled to carrier through reaction with sidechain amino groups in protein-based carriers, optionally followed by reduction of imino linkage formed.
30 The COX residue wherein X=OH can also be reacted with sidechain amino groups using condensing agents such as dicyclohexyl carbodiimide or other carbodiimide

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dehydrating agents. Linker compounds can also be used to effect the coupling; both homobifunctional and heterobi-functional linkers are available from Pierce Chemical Company, Rockford, IL. Compounds 31-34 described in the 5 examples below are designed to be coupled to antigenically neutral carriers through their C-terminal carboxyl groups (or amino groups in compound 32) using appropriate coupling agents.

The resulting immunogenic complex can then be 10 injected into suitable mammalian subjects such as mice, rabbits, and the like. Suitable protocols involve repeated injection of the immunogen in the presence of adjuvants according to a schedule which boosts production of antibodies in the serum. The titers of the immune 15 serum can readily be measured using immunoassay procedures, now standard in the art, employing the invention compounds as antigens.

The antisera obtained can be used directly or 20 monoclonal antibodies may be obtained by harvesting the peripheral blood lymphocytes or the spleen of the immunized animal and immortalizing the antibody-producing cells, followed by identifying the suitable antibody producers using standard immunoassay techniques.

The polyclonal or monoclonal preparations are 25 then useful in monitoring therapy or prophylaxis regimens involving the compounds of the invention. Suitable samples such as those derived from blood, serum, urine, or saliva can be tested for the presence of the administered inhibitor at various times during the 30 treatment protocol using standard immunoassay techniques which employ the antibody preparations of the invention.

The invention compounds can also be coupled to labels such as scintigraphic labels, e.g., technetium 99 or I-131, using standard coupling methods. The labeled

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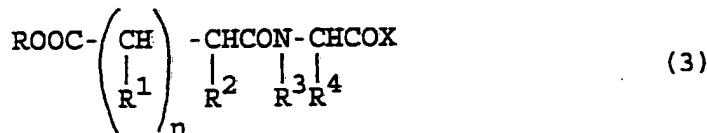
compounds are administered to subjects to determine the locations of excess amounts of one or more matrix metalloproteases *in vivo*. The ability of the inhibitors to selectively bind matrix metalloprotease is thus taken advantage of to map the distribution of these enzymes *in situ*. The techniques can also, of course, be employed in histological procedures and the labeled invention compounds can be used in competitive immunoassays.

10 Use As Affinity Ligands

The invention compounds can be coupled to solid supports, such as separation membranes, chromatographic supports such as agarose, sepharose, polyacrylamide, and the like, or to microtiter plates to obtain affinity supports useful in purification of various mammalian matrix metalloproteases. The selective binding of the matrix metalloproteases to the inhibitor ligand permits the adsorption of the desired enzyme and its subsequent elution using, for example, altered ionic strength and/or pH conditions.

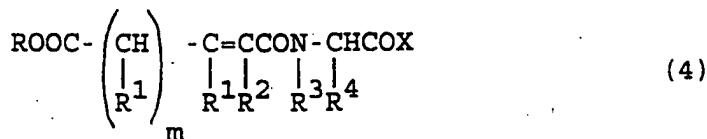
Preparation of the Invention Compounds

In general, the invention compounds that are hydroxamates are obtained by converting a carboxylic acid or ester precursor of the formulas



30 or

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5 wherein R is H or alkyl (1-6C) to the corresponding hydroxamates by treating these compounds or their activated forms with hydroxylamine under conditions which effect the conversion.

With respect to starting materials, the
10 components forming the $-\text{NR}^3-\text{CHR}^4\text{COX}$ moiety are readily available in the case of tryptophan and its analogs as esters or amides. As set forth above, many analogous fused bicyclo aromatic amino acids are described by Greenstein and Winitz (*supra*). Amino acids corresponding
15 to those wherein R^4 is 1-(2-methyl naphthyl)methylene; 1-quinolyl-methylene; 1-naphthyl methylene; 1-isoquinolyl methylene; and 3-isoquinolyl methylene can be prepared from the bicyclo aromatic methylene halides using the acetamido malonic ester synthesis of amino acids, as is
20 well understood in the art. The methylene halides themselves can be prepared from their corresponding carboxylic acids by reduction with lithium aluminum hydride and bromination of the resulting alcohol with thionyl bromide.

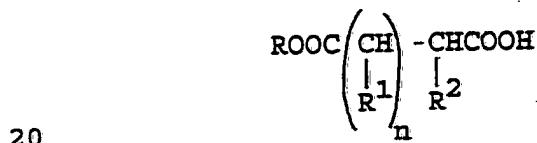
25 In general, the hydroxylamine reagent is formed in situ by mixing the hydroxylamine hydrochloride salt with an excess of KOH in methanol and removing the precipitated potassium chloride by filtration. The filtrate is then stirred with the precursor activated
30 carboxylic acid or ester of formula 3 or 4 for several hours at room temperature, and the mixture is then evaporated to dryness under reduced pressure. The residue is acidified, then extracted with a suitable organic solvent such as ethyl acetate, the extract washed

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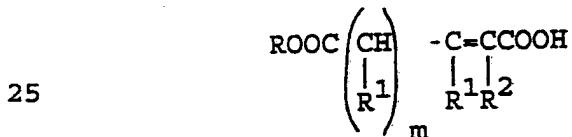
with aqueous potassium bisulfate and salt, and then dried with a solid drying agent such as anhydrous magnesium sulfate. The extract is then again evaporated to dryness and crystallized.

5 The substituted forms of the hydroxamate which include -NHOR⁷ are synthesized in an analogous manner but substituting H₂NOR⁷, wherein R⁷ is lower alkyl or acyl (1-4C) for hydroxylamine per se. The resulting O-alkyl or acyl hydroxamate can then be further alkylated, if
10 desired, to obtain the R⁷ONR⁶- derivative of the carboxylic acid. Similarly, HNR⁶OH may be reacted with the carboxylic acid to obtain the HONR⁶- derivative. HNCH₃OH and H₂NOCH₃ are commercially available.

15 To prepare the starting materials of formulas 3 and 4, the monoesterified carboxylic acid of the formula



or of the formula



is reacted with the acid of the formula



30 wherein X is other than OH under conditions wherein the condensation to form the amide bond occurs. Such conditions typically comprise mixture of the two components in a nonaqueous anhydrous polar aprotic solvent in the presence of base and a condensing agent such as a

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carbodiimide. Thus, the formation of the amide linkage can be catalyzed in the presence of standard dehydration agents such as the carbodiimides, for example dicyclohexyl carbodiimide, or N, N-carbonyl diimidazole. The 5 product is then recovered as a mixture of diastereomers of formula 3 or 4. This mixture is preferably used for the conversion to the hydroxamate and one of the resulting diastereomers is crystallized directly from the product mixture. Alternatively, the diastereomers are 10 separated by flash chromatography before conversion to the hydroxamate and recovered separately. This process is less preferred as compared to the process wherein separation of the diastereomers is reserved until the final product is obtained.

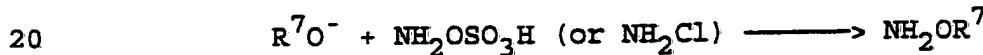
15 In the notation used in the examples, the "A" isomer is defined as that which migrates faster on TLC; the "B" isomer as that which migrates more slowly. When the "L" form of tryptophan or other amino acid containing a fused bicycloaromatic ring system is used as the 20 residue, and R¹ is H, in general, the "A" form is that which contains the corresponding configuration at the carbon containing the R² substituent (providing that is the only other center of asymmetry) in the final hydroxamate product. However, in Example 2, below, where 25 D-tryptophan is included in the composition, the "B" isomer contains what would correspond to an "L" configuration at the carbon containing R² in the compounds of formula 1.

When R⁶ and/or R⁷ = alkyl, the corresponding O- 30 or N-alkyl hydroxylamine is reacted with the methyl ester 4A as performed for unsubstituted hydroxylamine in Example 1. Alternatively, the methyl ester 4A can be saponified to its corresponding carboxylic acid and activated with oxalyl chloride or other condensing agent.

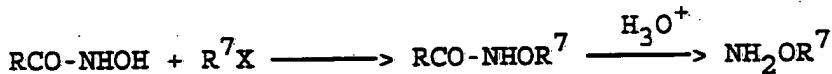
-20-

The alkyl hydroxylamine can then be reacted with the activated carboxylic acid to give the O- or N-substituted hydroxamic acid. O- and N-methylhydroxylamine can be purchased from the Aldrich Chemical Company.

5 Other N-alkyl hydroxylamines can be synthesized by conversion of aliphatic aldehydes to their oximes, followed by reduction to the N-alkyl hydroxylamine with borane-pyridine complex in the presence of 6N HCl (Kawase, M. and Kikugawa, Y.J., Chem Soc. Perkin Trans
10 (1979) 1:643. Other O-alkyl hydroxylamines can be synthesized by the general methods given by Roberts, J.S., "Derivatives of Hydroxylamine," Chapter 6.4 in Barton, D., et al., eds., Comprehensive Organic Chemistry (1979) 2:187-188 (Pergamon Press, Oxford). The two
15 general methods employed are displacement by R^7O^- of a leaving group from hydroxylamine sulfonic acid or chloramine, and O-alkylation of a hydroxamic acid with R^7-X followed by hydrolysis:



or



25 For R^7 = acyl, a hydroxamic acid of this invention can be acylated with an acid chloride, anhydride, or other acylating agent to give the compounds of this class (see Examples 19 and 20).

30 In some cases the derivatized maleic and succinic acid residues required for synthesis of the invention compounds are commercially available. If not, these can readily be prepared, in embodiments wherein R^1 is H or alkyl (1-8C) by reaction of a 2-oxocarboxylic ester of the formula $R^2COOCOR'$ in a Wittig reaction with 35 an alkyl triphenylphosphoranylidene acetate or α -

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triphenylphosphoranylidene alkanoate. The methyl acetate or alkanoate is preferred, but any suitable ester can be employed. This reaction is conducted in a nonaqueous, nonpolar solvent usually at room temperature. The
5 resultant compound is of the formula $\text{ROOCR}^1=\text{CR}^2\text{COOR}'$, wherein R and R' are residues of esterifying alkyl or arylalkyl alcohols.

If the compounds of formula 4 are desired, this product is condensed with the appropriate tryptophan or
10 analogous derivative; if the compounds of formula 3 are desired, the intermediate is reduced using hydrogen with a suitable catalyst. The sequence of reactions to obtain those embodiments wherein R^1 is H or alkyl, n is 1 and m is 0, and R^2 is alkyl are shown in Reaction Scheme 1.
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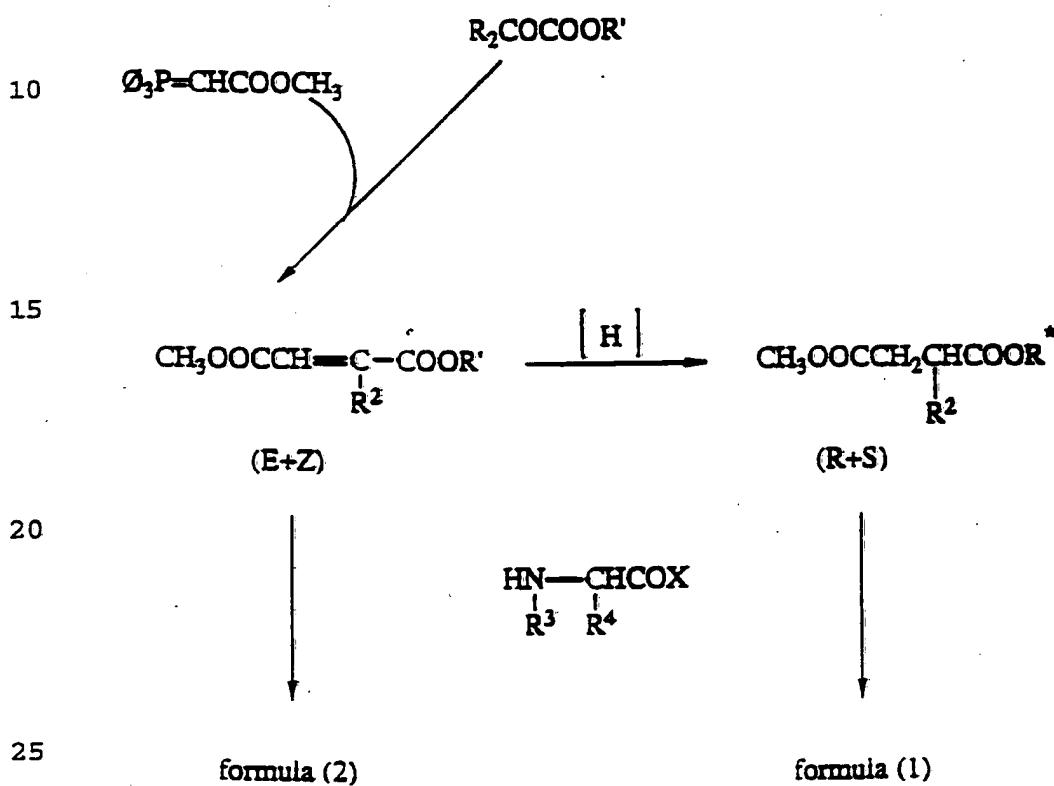
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Reaction Scheme 1

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* The hydrogenation reaction will remove R' when R' = benzyl.

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For those embodiments wherein R¹ and R² taken together are (CH₂)_p, the compounds of the invention are prepared analogously to the manner set forth in Reaction Scheme 1, except that the intermediate of the formula 5 ROOCCHR¹CHR²COOH is prepared from the corresponding 1,2-cycloalkane dicarboxylic acid--i.e., 1,2-cyclopentane dicarboxylic acid anhydride; 1,2-cyclohexane dicarboxylic anhydride or 1,2-cycloheptane dicarboxylic anhydride.

For compounds wherein -CONR³- is in modified 10 isosteric form, these forms can be prepared by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone 15 Modifications" (general review); Spatola, A.F., in "Chemistry and Biochemistry of Amino Acids Peptides and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general review); Hudson, 20 D., et al., Int J Pept Prot Res (1979) 14:177-185 (-CH₂NR³-, -CH₂CHR³-); Spatola, A.F., et al., Life Sci (1986) 38:1243-1249 (-CH₂-S); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 (-CH-CR³-, cis and trans); Almquist, R.G., et al., J Med Chem (1980) 23:1392-1398 25 (-COCHR³-); Jennings-White, C., et al., Tetrahedron Lett (1982) 23:2533 (-COCHR³-); Szelke, M., et al., European Application EP 45665 (1982) CA:97:39405 (1982) (-CH(OH)CHR³-); Holladay, M.W., et al., Tetrahedron Lett (1983) 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J., Life 30 Sci (1982) 31:189-199 (-CH₂-S-).

Preferred compounds of the invention include:

HONHCOCH₂CH(n-hexyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(n-pentyl)-CO-L-Trp-NHMe;

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- HONHCOCH₂CH(i-pentyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₃;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₂OH;
5 HONHCOCH₂CH(ethyl)-CO-L-Trp-NHcyclohexyl;
MeONHCOCH₂CH(iBu)-CO-L-Trp-NHET;
EtONMeCOCH₂CH(iBu)-CO-L-Trp-NHET;
MeONHCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHET;
10 EtONMeCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHET;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
HONHCOCH₂CH(i-Bu)CO-L-N-MeTrp-NHMe;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₂OH;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(S)CHMePh;
15 HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₆NH-CBZ;
HONHCOCH₂CH(i-Bu)CO-L-Ala(2-naphthyl)NHMe;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₄CH₃;
HONHCOCH₂CH(i-Bu)CO-L-Trp-piperidine;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₁₁CH₃;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NHcyclohexyl;
20 HONHCOCH₂CH(i-Bu)-L-Trp-OH;
HONMeCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
HONETCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
CH₃COONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
ΦCOONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
25 CH₃COONMeCOCH₂CH(i-Bu)CO-L-Trp-NHMe; and
ΦOCOONETCOCH₂CH(i-Bu)CO-L-Trp-NHMe.

The following examples are intended to illustrate but not to limit the invention.

30

EXAMPLES

In the examples below, TLC solvent systems are as follows: (A) ethyl acetate/methanol (95:5); (B) ethyl acetate/methanol (25:5); (C) ethyl acetate; (D) ethyl

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acetate/methanol (30:5); (E) ethyl acetate/hexane (1:1);
(F) chloroform/methanol/acetic acid (30:6:2); (G)
chloroform/methanol/acetic acid (85:10:1).

5

Example 1

Preparation of N-[D,L-2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-tryptophan methylamide

A suspension of 5 g (0.033 mol) of the sodium salt of 4-methyl-2-oxopentanoic acid and 5.65 g (0.033 mol) of benzyl bromide in 10 ml of anhydrous dimethylformamide was stirred for 4 days at room temperature. After evaporation of the solvent under reduced pressure the residue was diluted to 100 ml with hexane and washed with water (3 x 20 ml) and saturated sodium chloride and dried over anhydrous magnesium sulfate. Evaporation of solvent gave 6.4 g (88% yield) of the benzyl ester of 4-methyl-2-oxopentanoic acid (1) as a colorless oil.

A mixture of 6.4 g (0.029 mol) of (1) and 9.7 g (0.029 mol) of methyl(triphenylphosphoranylidene)acetate in 100 mL of dry methylene chloride was stirred for 12 hr at room temperature and evaporated to dryness. The residue was extracted with hexane (3 x 50 mL). The hexane solution was washed with 10% sodium bicarbonate (2 x 30 mL), water and saturated sodium chloride and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 8.01 g (100% yield) of benzyl 2-isobutyl-3-(methoxycarbonyl)-propionate (2) as a mixture of E and Z isomers.

A mixture of 8.01 g (0.029 mol) of (2) and 1 g of 10% palladium on carbon in 50 mL of methanol was hydrogenated at room temperature under 4 atmospheres of hydrogen gas for 8 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness under

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reduced pressure to give 4.7 g (86% yield) of 2-isobutyl-3-(methoxycarbonyl)-propionic acid (3) as a colorless oil.

To a mixture of 0.85 g (4.5 mmol) of (3) and 5 0.57 g (4.5 mmol) of oxalyl chloride in 10 mL of dry methylene chloride 0.1 mL of anhydrous dimethylformamide was added. After stirring for 1 hr at room temperature the solvent was evaporated under reduced pressure and the residue was diluted to 5 mL with anhydrous dimethylformamide and 10 1.06 g (4.1 mmol) of the hydrochloride salt of L-tryptophan methylamide (Kortylewicz and Galardy, J Med Chem (1990) 33:263-273) was added followed by addition of 1.3 mL (9.3 mmol) of triethylamine at -10°C. This was stirred for 7 hr at room temperature and evaporated to 15 dryness at room temperature under reduced pressure. The residue was diluted to 150 mL with ethyl acetate and washed with water (2 x 15 mL), 10% potassium bisulfate (5 x 20 mL), 10% sodium bicarbonate (2 x 20 mL), saturated sodium chloride and dried over anhydrous magnesium sulfate and then evaporated to give 1.6 g 20 (83% yield) of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-tryptophan methylamide 4 as a mixture of diastereomers, 4A and 4B.

Isomers 4A and 4B were separated by flash chromatography (silica gel, ethyl acetate).

Isomer 4A: mp=134-137°C. $R_f(C)=0.37$.

Isomer 4B: mp=156-158°C. $R_f(C)=0.2$.

Alternatively, the mixture of 4A and 4B was converted directly to its hydroxamate as described below. 30 In this case, 5A was crystallized from the mixture of 5A and 5B.

A warm mixture of 0.22 g (3.96 mmol) of potassium hydroxide in 1 mL of methanol was added to a warm mixture of 0.184 g (2.65 mmol) of the hydrochloride

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salt of hydroxylamine. After cooling in ice under an argon atmosphere the potassium chloride was filtered off and 0.5 g (1.32 mmol) of (4A) was added to the filtrate. The resulting mixture was stirred for 7 hr at room 5 temperature and evaporated to dryness under reduced pressure. The residue was suspended in 100 mL of ethyl acetate and washed with 10 mL of 10% potassium bisulfate, saturated sodium chloride and dried over anhydrous magnesium sulfate and evaporated to dryness under reduced 10 pressure. The residue was crystallized from ethyl acetate to give 0.28 g (56% yield) of pure 5A.

Isomer 4B was converted to its corresponding hydroxamic acid 5B (72% yield) as described for 4A.

Isomer 5A: mp=176-182°C. $R_f(D)=0.45$.

15 Isomer 5B: mp=157-162°C. $R_f(D)=0.39$.

For the case wherein the 4A/4B mixture is used, the 5A can be crystallized directly from the residue as described above.

In a similar manner to that set forth above, 20 but substituting for 4-methyl-2-oxopentanoic acid, 2-oxopentanoic acid, 3-methyl-2-oxobutyric acid, 2-oxohexanoic acid, 5-methyl-2-oxohexanoic acid, or 2-decanoic acid, the corresponding compounds of formula 1 are prepared wherein R^1 is H and R^2 is an n-propyl, i-propyl, 25 n-butyl, 2-methylbutyl, and n-octyl, respectively. In addition, following the procedures set forth hereinabove in Example 1, but omitting the step of hydrogenating the intermediate obtained by the Wittig reaction, the corresponding compounds of formula 2 wherein R^1 is H and R^2 is 30 as set forth above are obtained.

To synthesize the compounds containing acylated forms of the indolyl residue, the intermediate ester of formula 3 or 4 is deesterified and acylated prior to conversion to the hydroxamate. For illustration, 4A is

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deesterified with sodium hydroxide in ethanol and then acidified to give N-(L-2-isobutyl-3-carboxypropanoyl)-L-tryptophan methylamide, which is treated with the anhydride of an alkyl (1-4C) carboxylic acid to obtain
5 N-(L-2-isobutyl-3-carboxypropanoyl)-L-((N-acyl)indolyl)-tryptophan methylamide. This intermediate is then treated with oxalyl chloride followed by hydroxylamine at low temperature to give the corresponding hydroxamate.

10

Example 2

Preparation of N-[2-isobutyl-3-(N'-hydroxy-carbonylamido)-propanoyl]-D-tryptophan methylamide (7B)

The mixture of the two diastereoisomers of N-[2-isobutyl-3-(methoxycarbonyl)-propanoyl]-D-tryptophan methyl amide 6A,B was prepared as described for 4A,B in Example 1. The mixture was crystallized from ethyl acetate to give, after two recrystallizations, 0.26 g (49%) of the pure diastereomer 6B: mp 155-157°C, R_f(C)=0.32. 6B was converted into its hydroxamic acid 7B by the method described in Example 1 in 50% yield (119 mg): mp 157-159°C, R_f(D)=0.39.

Example 3

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl-amido)-propanoyl]-N-methyl-L-tryptophan methylamide (9A)

The reaction of N-methyl-L-tryptophan-methylamide, prepared as described in Example 1 for L-tryptophan methylamide, with 3 performed as described for 4 gave crude N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-N-methyl-L-tryptophan methylamide 8A,B which was crystallized from ethyl acetate to give 76 mg (19% yield) of 8A: mp 171-174°C, R_f(C)=0.40.

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8A was converted into 9A by the method described in Example 1 in 45% yield (34 mg): mp 180-183°C, $R_f(D)=0.54$.

5

Example 4

Preparation of N-[2-isobutyl-3-(N-hydroxycarbonyl amido)-propanoyl]-L-3-(2-naphthyl)-alanine methylamide (11A)

N-[D,L-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-3-(2-naphthyl)-alanine 10A was prepared as described in 10 Example 1 from L-3-(2-naphthyl)-alanine methylamide and 3. The crude product was chromatographed on 60 g of silica gel in ethyl acetate:hexane 1:1 to yield 12 mg (5% yield) of 10A: mp 151-158°C, $R_f(C)=0.69$.

10A was converted into the hydroxamate 11A as 15 in Example 1 in 30% yield (3 mg): mp 179-181°C, $R_f(D)=0.17$. MS-FAB (m/z) 400 ($M^+ +H$) .

Example 5

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl amido)-propanoyl]-L-tryptophan 2-hydroxyethylamide (13A)

The hydrochloride salt of L-tryptophan 2-hydroxyethylamide was prepared and coupled with 3 as described for the hydrochloride salt of L-tryptophan methylamide in Example 1 except that 3 was activated with 25 1,1'-carbonyldiimidazole for 20 minutes in methylene chloride at room temperature. The crude product was a mixture of 0.7 g (67% yield) of the diastereoisomers 12A,B: $R_f(C)$ 12A 0.38, $R_f(C)$ 12B 0.19.

12A crystallized from ethyl acetate in 35% 30 yield (0.18 g): mp 161-163°C, $R_f(C)=0.38$.

12A was converted into N-[2-isobutyl-3-(N'-hydroxycarbonylamido)-propanoyl]-L-tryptophan 2-hydroxyethylamide 13A as in Example 1 in 35% yield (62 mg): $R_f(D)=0.17$, mp 162-163°C. MS-FAB (m/z) 419 ($M^+ +H$) .

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Example 6

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl amido)-propanoyl]-L-tryptophan amylamide (15A)

5 The hydrochloride salt of L-tryptophan amylamide was prepared as described in Example 1 for L-tryptophan methylamide and was reacted with 3 that had been activated with 1,1'-carbonyldiimidazole for 20 minutes in dichloromethane at room temperature. The
10 mixture of the two diastereomers of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-tryptophan amylamide 14A,B (90% yield) was converted to its corresponding hydroxamic acids as described for 4A. Slow evaporation of the ethylacetate solution gave 0.343 g (71%) of 15A,B: mp
15 160-163°C. MS-FAB (m/z) 445 ($M^+ + H$).

Example 7

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl amido)-propanoyl]-L-tryptophan piperidinamide (17A,B)

20 L-tryptophan piperidinamide was reacted with 3 as performed in Example 1 for L-tryptophan methylamide to give 1.14 g (89% yield) of N-[D,L-2-isobutyl-3-(methoxycarbonyl)-propanoyl]-L-tryptophan piperidinamide 16A,B as a foam; R_f (C) (16A) 0.74, (16B) 0.67.
25 16A,B was converted into crude 17A,B identically to 4A in Example 1 in 88% yield (570 mg): R_f (D) (17A) 0.41, (17B) 0.30. Crude 17A,B was chromatographed on 180 g of silica gel in 12% isopropanol in ethyl acetate to give 140 mg (25% yield) of 17A,B
30 after crystallization from ethyl acetate: mp 169-170°C. MS-FAB (m/z) 443 ($M^+ + H$).

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Example 8

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl
amido)-propanoyl]-L-tryptophan dodecylamide (19A)

The reaction of L-tryptophan dodecylamide was
5 prepared in a manner analogous to that described for
L-tryptophan methylamide in Example 1. This ester was
reacted with 3 as described in Example 1 to give crude
N-[D,L-isobutyl-3-(methoxycarbonyl)-propanol]-L-
tryptophan dodecylamide 18A,B in 93% yield as a mixture
10 of isomers 19A and 19B. This mixture was chromatographed
on 150 g of silica gel in ethyl acetate:hexane, 1:2, to
yield 0.62 g of the mixture of the two isomers: $R_f(E)$
19A 0.37, $R_f(E)$ 19B 0.29.

Crystallization by slow evaporation from ethyl
15 acetate gave 0.38 g of 18A contaminated by approximately
10% of 18B by TLC and NMR analysis: mp 133-135°C. 18A
was converted to its corresponding hydroxamic acid as
described in Example 1, except that the potassium salt of
19A crystallized from the alkaline reaction mixture in
20 81% yield (222 mg). The potassium salt of 19A (54 mg)
was dissolved in 2 mL of boiling methanol, a few drops of
water were added, and the solution was acidified to pH 6
with 0.1 N hydrochloric acid and diluted with water to
give 50 mg (100% yield) of 19A: mp 155-159°C,
25 $R_f(D)=0.49$. MS-FAB (m/z) 543 ($M^+ + H$).

Example 9

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonylamido)
propanoyl]-L-tryptophan (S)-methylbenzylamide (21A)

30 The reaction of L-tryptophan (S)-methylbenzyl-
amide with 3 was performed as described in Example 1 to
give, after crystallization from ethyl acetate, 330 mg
(51% yield) of N-[2-isobutyl-3-(methoxycarbonyl)-

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propanoyl]-L-tryptophan (S)-methylbenzylamide 20A: mp
160-162°C, R_f(C)=0.77.

20A was converted into hydroxamate 21A by the identical method used in Example 1 in 38% yield (76 mg):
5 mp 165-166°C, R_f(D)=0.73. MS-FAB (m/z) 479 (M⁺ + H).

Example 10

Preparation of N-[L-2-isobutyl-3-(N'-hydroxy-carbonylamido)-propanoyl]-L-tryptophan (6-phenyl-methoxycarbonylamino-hexyl-1)amide (27A)

To prepare 1-amino-6-phenylmethoxycarbonyl-amino-hexane (23), an equimolar mixture (0.01 mol) of 1,6-diaminohexane and benzaldehyde in 25 mL of methylene chloride was stirred for 5 hr in the presence of 1.5 g of anhydrous magnesium sulfate at room temperature. After removing the drying agent by filtration the filtrate was evaporated to dryness under reduced pressure to give 2 g (100% yield) of crude 1-amino-6-phenylamino-hexane 22 as a colorless oil; NMR(CDCl₃) 1.1 - 1.9 (m, 10H, hexane CH₂-2,-3,-4,-5, NH₂); 2.6 (m, 2H, CH₂-1); 3.51 (m, 2H, hexane CH₂-6); 7.1-7.8 (m, 5H, aromatic); 8.16 (s, 1H, imine CH). To a mixture of 2 g (0.01 mol) of 22 and 1.4 mL (0.01 mol) of triethylamine in 20 mL of methylene chloride. Then 1.78 g (0.01 mol) of benzylchloroformate was added dropwise at -5°C. The resulting mixture was stirred for 0.5 hr at 0°C and for 2 hr at room temperature then diluted to 50 mL with methylene chloride and washed with water (20 ml), 2% sodium bicarbonate (20 ml), water and saturated sodium chloride and dried over anhydrous magnesium sulfate. After evaporation of solvent under reduced pressure the residue was dissolved in 5 mL of ethanol and 10 mL of 2N hydrochloric acid was added. The resulting mixture was stirred for 6 hr at room temperature then evaporated to dryness under reduced

-33-

pressure. The residue was diluted to 50 mL with water and washed with ethyl ether (2 x 15 ml). The water phase was evaporated under reduced pressure and the product 23 was purified by crystallization from a small portion of 5 water with a yield of 42%; mp 175-178°C.

To prepare the dipeptide analog '(N-(L-2-isobutyl-3-methoxycarbonyl)-propanoyl-L-tryptophan (25A)), for derivatization to 23: To a mixture of 1.754 g (9.32 mmol) of 2-isobutyl-3-methoxycarbonylpropionic acid 3 in 4 mL of 50% anhydrous DMF in methylene chloride 10 1.66 g (10.2 mmol) of N,N'-carbonyldiimidazole (CDI) was added at room temperature. After 15 minutes of stirring at room temperature, 3.08 g (9.31 mmol) of the hydrochloride salt of L-tryptophan benzyl ester was added. 15 The resulting mixture was stirred overnight at room temperature, then diluted to 60 mL with ethyl acetate and washed with 5% sodium bicarbonate (2 x 15 ml), water (2 x 15 ml), saturated sodium chloride solution and dried over magnesium sulfate. Evaporation of the solvent under 20 reduced pressure gave 4.32 g (100% yield) of 24, the benzyl ester of 25 as a colorless foam, which was used in the next step without further purification.

Hydrogen gas was bubbled through a mixture of 4.32 g (9.31 mmol) of 24 and 0.5 g of 10% palladium on 25 carbon in 15 mL of methanol for 2 hr while methanol was added to keep the volume of the reaction mixture constant. The catalyst was filtered off and washed with a fresh portion of methanol (15 ml) and the filtrate was evaporated to dryness under reduced pressure. Evaporation of the solvent under reduced pressure and drying of the residue in vacuo gave 3.08 g (88% yield) of acid 30 25A,B as a mixture of two diastereoisomers, in the form of a colorless glassy solid. This was separated to give

-34-

isomers 25A and 25B by flash chromatography (silica gel; ethyl acetate; R_f (25A)=0.24, R_f (25B)=0.1).

The compound 25A was converted to N-[L-2-isobutyl-3-methoxycarbonylpropanoyl]-L-tryptophan (6-phenylmethoxycarbonylamino-hexyl-1)amide (26A) as follows. A mixture of 0.55 g (1.47 mmol) of 25A and 0.24 g (1.48 mmol) of CDI in 1 mL of 2% dimethylformamide in methylene chloride was stirred for 0.5 hr at room temperature and 0.42 g (1.47 mmol) of 23 was added.

After stirring overnight at room temperature, the mixture was diluted to 50 mL with chloroform and washed with 2% potassium bisulfate (2 x 10 ml), water (10 ml), 5% sodium bicarbonate (2 x 10 ml), water (2 x 10 ml) and saturated sodium chloride and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure gave 0.8 g of the crude 26A which was purified by flash chromatography (silica gel; ethyl acetate/hexane 25:5): Yield 56%; R_f (E)=0.57.

When the product 26A is substituted for 4A in Example 1, the identical process afforded the title compound 27A, melting at 102-108°C, in 46% yield; R_f (D)=0.63.

Example 11

Preparation of N-[L-2-isobutyl-3-(N'-hydroxycarbonyl-amido)-propanoyl]-L-tryptophan cyclohexylamide (28A)

When cyclohexylamine is substituted for 23 in Example 10, the identical process afforded the title compound 28A melting at 199-203°C, in 49% yield;

R_f (D)=0.51.

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Example 12

Preparation of N-[cis-2-(N'-hydroxycarbonyl-amido)-cyclohexylcarbonyl-L-tryptophan methylamide (29A,B)

A mixture of 2 g (0.013 mol) of cis-1,2-
5 cyclohexane-dicarboxylic anhydride in 15 mL of methanol
was refluxed for 5 hr, then evaporated to dryness under
reduced pressure to give 2.41 g (100% yield) of cis-2-
methoxycarbonyl-cyclohexanecarboxylic acid. When this
was substituted for 3 in Example 1, the identical process
10 afforded the title compound, melting at 140-144°C, in 36%
yield; $R_f(D)=0.53, 0.47$.

Example 13

Preparation of N-[trans-2-(N'-hydroxycarbonyl-amido)-cyclohexylcarbonyl-L-tryptophan methylamide (30A,B)

When (\pm)trans-1,2-cyclohexanedicarboxylic
anhydride was substituted for cis-1,2-cyclohexane-
dicarboxylic anhydride in Example 12, the identical
process afforded the title compound 30A,B, melting at
20 167-174°C, in 37% yield; $R_f(D)=0.57$.

Example 14

Preparation of N-[2-isobutyl-3-(N'-hydroxycarbonyl-amido)-propanoyl]-L-tryptophan (31A)

31A was prepared from 25A in Example 10 in a
similar manner to the preparation of 5A in Example 1 in
75% yield (128 mg) and isolated as a foam from ethyl
acetate: $R_f(F)=0.55$, MS-FAB (m/z) ($M^+ + H$). A small
sample of 31A recrystallized from ethyl acetate had a
30 melting point of 116-120°C.

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Example 15

Preparation of N-(D,L-2-isobutyl-3-carboxypropanoyl)-L-tryptophan (6-aminohexyl-1)amide (32A)

A mixture of 0.5 g (8.24 mmol) of 26A in 0.4 mL
5 of 2N potassium hydroxide in methanol was stirred
overnight at room temperature, then evaporated to dryness
under reduced pressure. The residue was diluted to 15 mL
with water and acidified to pH = 2 with 1N hydrochloric
acid. The crude free acid of 26A was taken up with ethyl
10 acetate (3 x 15 ml) and the organic phase was dried over
anhydrous magnesium sulfate and evaporated to dryness to
give 0.45 g (92% yield) of 26A as a colorless foam.

Hydrogen gas was bubbled through a mixture of
0.395 g (6.6 mmol) of the free acid of 26A in 15 mL of
15 methanol for 2 hr, in the presence of 0.12 g of 10%
palladium on carbon at room temperature. The catalyst
was filtered off, washed with ethanol (2 x 20 ml) and the
filtrate was evaporated to dryness under reduced pressure
to give 0.3 g (92% yield) of the title compound 32A as a
20 colorless foam; $R_f(G) = 0.08$.

Example 16

Preparation of N-[N-(2-isobutyl-3-carboxypropanoyl)-L-tryptophanyl] glycine 34A,B

The reaction of L-tryptophanyl-glycine methyl
ester with acid 3, performed as described for 25A gave
crude N-[N-(D,L-2-isobutyl-3-methoxycarbonylpropanoyl)-
L-tryptophanyl]-glycine methyl ester 33 in 87% yield as a
mixture of diastereomers 33A and 33B. Isomers 33A and
30 33B were separated by flash chromatography (silica gel;
ethyl acetate). Isomer 33A mp = 154-155°C; $R_f(C) = 0.46$.

Esters 33A,B were transformed to free acids
34A,B by saponification with two equivalent of methanolic

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potassium hydroxide, as described for 25A. Isomer 34A yield 92%; mp = 96-102°C; R_f(G) = 0.31.
Isomer 34B yield 93%; mp = 99-105°C;
R_f(G) = 0.25.

5

Example 17

Preparation of N-(cis-2-carboxy-cyclohexylcarbonyl)-L-tryptophan methylamide 35

To a mixture of 0.281 g (1.82 mmol) of cis-1,2-cyclohexanedicarboxylic anhydride and 0.47 g of the hydrochloride salt of L-Trp-NHMe in 0.5 mL of dimethylformamide 0.51 mL of triethylamine was added at room temperature. After 2 hr of stirring the resulting mixture was diluted to 10 mL with water and 25 mL of ethyl acetate was added. The resulting mixture was acidified to pH = 2 with 10% potassium bisulfate and the organic phase was washed with water (2 x 15 ml), saturated sodium chloride and dried over anhydrous magnesium sulfate and evaporated to dryness. The title compound 35 was purified by crystallization from an ethyl acetate-hexane mixture. Yield 48%; mp = 105-112°C; R_f(G) = 0.65, 0.61.

Example 18

25 Preparation of N-(trans-2-carboxy-cyclohexylcarbonyl)-L-tryptophan methylamide 36

When (+) trans-1,2-cyclohexanedicarboxylic anhydride is substituted for cis-1,2-cyclohexane-dicarboxylic anhydride in Example 17, the identical process afforded the title compound 36 in 56% yield: mp = 167-174°C; R_f(G) = 0.67, 0.61.

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Example 19

Preparation of N-[2-isobutyl-3-(N'-acetoxy carbonylamido)-propanoyl-L-tryptophan methylamide (37A)

To 97.5 mg (0.25 mmol) of 5A (Example 1) in 0.5 ml of dimethylformamide was added 25.5 mg (0.25 mmol) of acetic anhydride and 37 mg (0.25 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at room temperature. After standing overnight, the DMF was evaporated under high vacuum and the residue taken up in a mixture of equal volumes of ethyl acetate and 2% potassium bisulfate. The ethyl acetate layer was washed with 2% potassium bisulfate, water, and brine, dried over magnesium sulfate, and evaporated to give a solid. The solid was dissolved in a 1:1 mixture of hot ethyl acetate:hexane, which upon standing at room temperature gave 71 mg (66% yield) of solid product 37A: mp=184-186°C; R_f(G)=0.68.

Example 20

Preparation of N-[isobutyl-3-(N'-benzoxycarbonylamido)-propanoyl-L-tryptophan methylamide (38A)

To 30.5 mg (0.25 mmol) of benzoic acid in 1 ml of tetrahydrofuran was added 40.5 mg (0.25 mmol) of carboonyldiimidazole. After 10 minutes, 97 mg (0.25 mmol) of compound 5A from Example 1 was added in 1 ml of dimethylformamide. After 10 minutes, the reaction mixture was evaporated to dryness under high vacuum, and dissolved in a mixture of equal volumes of ethyl acetate and water. The ethyl acetate layer was washed with 5% sodium bicarbonate, water, 2% sodium bisulfate, water, and brine, and dried over magnesium sulfate. Evaporation of the ethyl acetate layer to a small volume gave 50 mg (41%) of the title compound, 38A: mp=187-187.5°C; R_f(G)=0.54.

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Example 21

Applying the methods set forth above, the following invention compounds are synthesized:

5

HONHCOCH₂CH(n-hexyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(n-pentyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(i-pentyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHMe;
10 HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₃;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₂OH;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHcyclohexyl;
MeONHCOCH₂CH(iBu)-CO-L-Trp-NHET;
EtONMeCOCH₂CH(iBu)-CO-L-Trp-NHET;
15 MeONHCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHET; and
EtONMeCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NHET.

Example 22

Assay of Inhibition Activity

20

Inhibitors were assayed against crude or purified human skin fibroblast collagenase using the synthetic thiol ester substrate at pH 6.5 exactly as described by Kortylewicz & Galardy, J Med Chem (1990) 33:263-273. The collagenase concentration was 1-2 nM.

25

The compounds of Examples 1-18 are tested for their ability to inhibit crude collagenase and gelatinase from human skin fibroblasts, crude collagenase and gelatinase from purulent human sputum in this assay. The results with respect to crude enzyme preparation are shown in

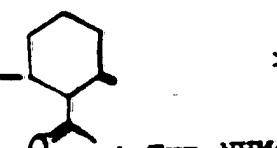
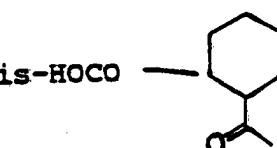
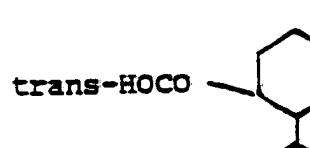
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Table 1. The Ki of 5A for purified human skin collagenase is 0.4 nM. Assays for inhibition of human stromelysin are conducted as described by Teahan, J., et al., Biochemistry (1989) 20:8497-8501.

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Table 1

| No. | Compound | K_i (nM) |
|-----|--|------------|
| 5 | 1 5A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHMe | 10 |
| | 1 5B NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHMe | 150 |
| | 2 7A NHOHCOCH ₂ CH(i-Bu)CO-D-Trp-NHMe | 70,000 |
| | 3 9A NHOHCOCH ₂ CH(i-Bu)CO-L-N-MeTrp-NHMe | 500 |
| | 4 11A NHOHCOCH ₂ CH(i-Bu)CO-L-Ala(2-naphthyl)NHMe | 15 |
| 10 | 5 13A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₂ OH | 20 |
| | 6 15A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₄ CH ₃ | 30 |
| | 7 17A,B NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-piperidine | 200 |
| | 8 19A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₁₁ CH ₃ | 300 |
| | 9 21A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(S)CHMePh | 3 |
| 15 | 10 27A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂) ₆ NH-CBZ | 13 |
| | 11 28A NHOHCOCH ₂ CH(i-Bu)CO-L-Trp-NHcyclohexyl | 50 |
| 20 | 12 29A,B cis-NHOHCO —  | >10,000 |
| | 13 30A,B trans-NHOHCO —  | >10,000 |
| 25 | 14 31A NHOHCO-CH ₂ CH(i-Bu)-L-Trp-OH | 200 |
| | 15 32A HOOC-CH ₂ CH(i-Bu)CO-L-Trp-NH(CH ₂)NH ₂ | >10,000 |
| | 16 34A HOOC-CH ₂ CH(i-Bu)CO-L-Trp-Gly-OH | >10,000 |
| | 34B HOOC-CH ₂ CH(i-Bu)CO-L-Trp-Gly-OH | >10,000 |
| 30 | 17 35 cis-HOOC —  | >10,000 |
| | 18 36 trans-HOOC —  | >10,000 |

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Example 21

Prevention of Corneal Ulceration in the
Alkali Burned Rabbit Cornea

The ability of the invention compounds to
5 prevent ulceration has been confirmed by a corneal assay
conducted by Gregory Schultz, University of Florida,
Gainesville, FL.

Twenty rabbit eyes were burned for 60 seconds
with 1 N NaOH to a diameter of 10 mm. The ten control
10 eyes were treated with two drops of hypotonic buffer
every two hours from 8 AM to 6 PM, and then with a
subconjunctival injection of 0.5 mL of buffer in the
evening. The experimental eyes were treated identically
but with 400 µg per mL inhibitor in buffer. Eyes were
15 scored clinically with 0 being no ulceration and 5
equalling perforation of the cornea. Figure 1 shows
average clinical score over 26 days. The compound 5A
shows marked protection of the cornea from perforation.
Figure 2 shows percent of corneas not perforated over 26
20 days; compound 5A shows 100% protection.

A histological examination of the treated and
untreated corneas showed the following:

Perpendicular sections though the corneas of
rabbits were examined 28 days following severe alkali
25 injuries which were made by exposing the corneas of
anesthetized rabbits to 2 N sodium hydroxide for 60
seconds in a 12.5 mm diameter well. Following injury,
rabbits were treated topically with 2 drops every other
hour between 8 AM and 6 PM followed by a subconjunctival
30 injection of 0.5 mL of collagenase inhibitor of formula
5A or vehicle (50 mM Hepes, Antibiotics).

The cornea of a rabbit treated with collagenase
inhibitor shows lamellae that have begun to repopulate
with keratocytes which have most likely migrated from the

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uninjured peripheral cornea and sclera. The stroma also contains some inflammatory cells presumably macrophages which have migrated into the injured cornea. There is little evidence for disruption of the extracellular
5 matrix of the stromal lamellae and there is no evidence of significant extracellular matrix destruction. The epithelium in this section has resurfaced the cornea, although the epithelium is tenuously attached as evidenced by the separation of the sections of the
10 epithelium from the underlying stroma. There is no evidence of neovascularization of this part of the cornea. The endothelial surface has not regenerated and Descemet's membrane has separated from the stroma in a cloudy cornea which lacks persistent and complete
15 epithelial regeneration and also lacks significant stromal ulceration.

Cornea treated only with vehicle shows a dominant appearance of extensive degradation of the stromal matrix and the presence of massive inflammatory
20 cell infiltrates. These inflammatory cells most probably are macrophages and neutrophils. The stromal lamellae have digested in this section approximately two-thirds of the complete depth of the stroma, and in adjacent sections, erosion has occurred to Descemet's membrane.
25 The stroma appears to have a frayed appearance at the edge where the inflammatory cell infiltration is most extensive. Fracture lines running through the stroma suggest a general weakening of the extracellular matrix. In this section of the cornea there is no evidence of
30 neovascularization, epithelial cells, or endothelial cells. Fragments of endothelial cells are present on Descemet's membrane together with inflammatory cells in the anterior chamber fluid. Few if any keratocytes can be identified in the stroma. This microscopic section is

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generally consistent with the results of slit lamp microscopy which indicated that the cornea had extensive ulceration and peripheral neovascularization.

Overall, the histopathology of these two
5 sections suggest that a major effect of the collagenase inhibitor in preventing ulceration is due to a reduction in the inflammatory cell filtration into the alkali injured cornea. Furthermore, this section suggests that repopulation of the stroma in the collagenase inhibitor
10 treated corneas has begun and incomplete epithelial regeneration of a transient nature has begun on the epithelial surface with no regeneration of the endothelium.

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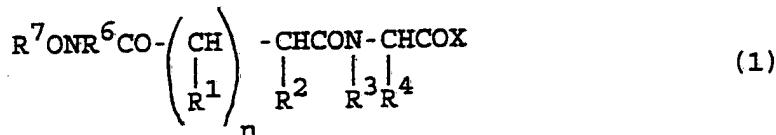
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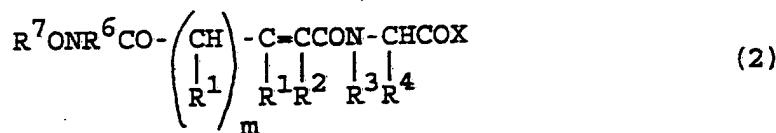
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Claims

1. A compound which inhibits at least one mammalian matrix metalloprotease, said compound having
 5 the formula:



10 or



15 wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(\text{CH}_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

20 R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

25 X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

X is the residue of a cyclic amine or hetero-

cyclic amine; and

30 wherein R^6 is H or lower alkyl (1-4C) and R^7 is H, lower alkyl (1-4C) or an acyl group, and wherein $-\text{CONR}^3-$ is optionally in modified isosteric form.

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2. The compound of claim 1 wherein R³ is H, and/or R⁷ and R⁶ are H, and/or R¹ is H and/or R² is alkyl (3-8C), and/or X is NHR⁵ or the residue of a cyclic amine or heterocyclic amine, and/or R⁴ is selected from the group consisting of 1-(2-methyl naphthyl)methylene; 1-quinolyl methylene; 1-naphthyl methylene; 2-naphthyl methylene; 1-isoquinolyl methylene; 3-isoquinolyl methylene; 3-thionaphthenyl methylene; 3-cumaronyl methylene; 3-(5-methylindolyl)methylene; 3-(5-hydroxyindolyl)methylene; 3-(2-hydroxyindolyl)methylene; biphenyl methylene; (3-indolyl)methylene and 4-phenylpyrimidyl methylene; and the substituted forms thereof.

15 3. The compound of claim 2 wherein X is NHR⁵ and wherein R⁵ is alkyl (1-4C); a substituted alkyl (1-4C substituted with one hydroxyl group); aryl alkyl; or a substituted alkyl (2-7C substituted with phenylmethoxy-carbonylamido), and/or R² is isobutyl, 2-methylbutyl, or 20 isopropyl, and/or R⁴ is (3-indolyl)methylene or its N-acylated (1-5C) substituted form.

25 4. The compound of claim 1 which is selected from the group consisting of:

HONHCOCH₂CH(n-hexyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(n-pentyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(i-pentyl)-CO-L-Trp-NHMe;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHMe;
30 HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₃;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHCH₂CH₂OH;
HONHCOCH₂CH(ethyl)-CO-L-Trp-NHcyclohexyl;
MeONHCOCH₂CH(iBu)-CO-L-Trp-NHET;
EtONMeCOCH₂CH(iBu)-CO-L-Trp-NHET;

35

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MeONHCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NH₂;
EtONMeCOCH₂CH(iBu)-CO-L-Ala(2-naphthyl)-NH₂;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
HONHCOCH₂CH(i-Bu)CO-L-N-MeTrp-NHMe;
5 HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₂OH;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(S)CHMePh;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₆NH-CBZ;
HONHCOCH₂CH(i-Bu)CO-L-Ala(2-naphthyl)NHMe;
10 HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₄CH₃;
HONHCOCH₂CH(i-Bu)CO-L-Trp-piperidine;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NH(CH₂)₁₁CH₃;
HONHCOCH₂CH(i-Bu)CO-L-Trp-NHcyclohexyl;
HONHCOCH₂CH(i-Bu)-L-Trp-OH;
15 HONMeCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
HONETCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
CH₃COONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
OCOONHCOCH₂CH(i-Bu)CO-L-Trp-NHMe;
CH₃COONMaCOCH₂CH(i-Bu)CO-L-Trp-NHMe; and
20 OCOCNtCOCH₂CH(i-Bu)CO-L-Trp-NHMe.

20 5. The compound of claims 1-4 conjugated to a moiety which is a radioactive label, an antigenically neutral carrier, a targeting ligand, or a solid support.

25 6. A pharmaceutical composition effective in treating conditions characterized by unwanted matrix metalloprotease activity, which composition comprises an amount of the compound of claims 1-4 effective to inhibit said matrix metalloprotease activity in admixture with a 30 pharmaceutically acceptable excipient.

35 7. The use of the compound of claims 1-4 or a pharmaceutical composition thereof to inhibit said matrix metalloprotease activity to treat conditions

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characterized by unwanted matrix metalloprotease activity
wherein said condition is selected from the group
consisting of stomach ulcers, superficial wounds,
epidermolysis bullosa, skin cancer, pemphigus, septic
5 shock and ARDS.

8. A method to inhibit matrix metalloprotease
activity in vitro or in cell culture, which method
comprises contacting the matrix metalloprotease to be
10 inhibited with an effective amount of the compound of
claims 1-4.

9. An antibody preparation specifically
immunoreactive with the compound of claims 1-4.
15

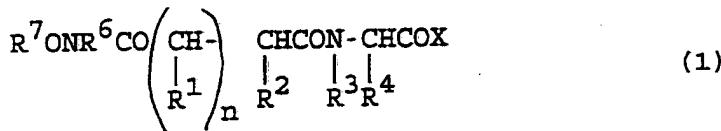
10. A method to assess the level of the
compound of claims 1-4 in a biological sample, which
method comprises contacting the sample with an antibody
preparation specifically immunoreactive with the compound
20 of claims 1-4 under conditions wherein said antibodies
complex with said compound if present, and
detecting the presence or absence of the
complex.

25 11. A method to prepare a compound of the
formula

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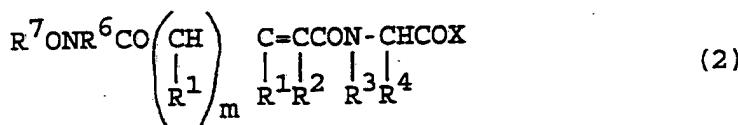
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or



10 wherein each R^1 is independently H or alkyl (1-8C) and R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2 taken together are $-(CH_2)_p-$ wherein $p = 3-5$;

R^3 is H or alkyl (1-4C);

15 R^4 is fused or conjugated unsubstituted or substituted bicycloaryl methylene;

n is 0, 1 or 2;

m is 0 or 1; and

20 X is OR^5 or NHR^5 , wherein R^5 is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

X is an amino acid residue or amide thereof; or

25 X is the residue of a cyclic amine or heterocyclic amine; and

wherein R^6 is H or lower alkyl (1-4C) and R^7 is

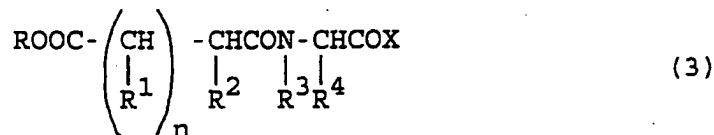
H, lower alkyl (1-4C) or an acyl group, and wherein $-CONR^3-$ is optionally in modified isosteric form;

which method comprises treating a compound of the formula

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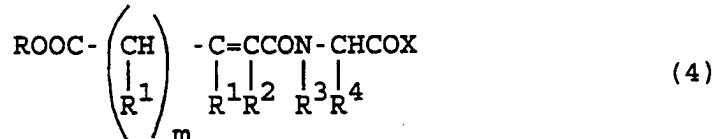
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or



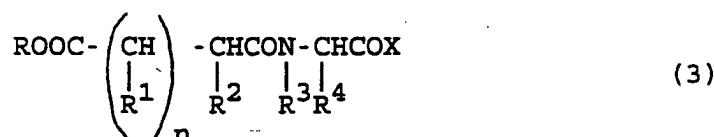
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or an activated form thereof;

wherein R is H or alkyl(1-6C), and the
 remaining substituents are as above defined, with an
 amount of substituted or unsubstituted hydroxylamine and
 under conditions effective to convert the compounds of
 15 the formulas (3) and (4) into the compounds of formulas
 (1) and (2), respectively, and
 recovering the compound of formula (1) or (2).

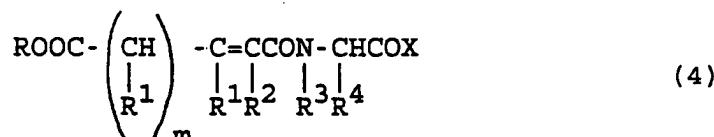
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12. A compound of the formula



25

or



30

wherein R is H or alkyl(1-6C);

each R^1 is independently H or alkyl (1-8C) and
 R^2 is alkyl (1-8C) or wherein the proximal R^1 and R^2
 taken together are $-(\text{CH}_2)_p-$ wherein p = 3-5;
 35 R^3 is H or alkyl (1-4C);

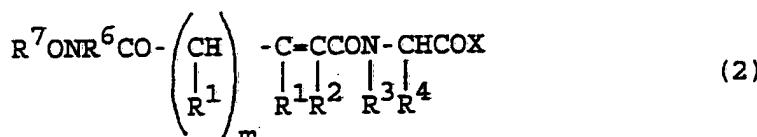
-50-

R⁴ is fused or conjugated unsubstituted or substituted bicycloaryl methylene;
 n is 0, 1 or 2;
 m is 0 or 1; and
 5 X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or
 X is an amino acid residue or amide thereof; or
 X is the residue of a cyclic amine or hetero-
 10 cyclic amine; and
 wherein -CONR³ is optionally in modified isosteric form.

13. A method to prepare a compound of the
 15 formula



20 or



25 wherein each R¹ is independently H or alkyl (1-8C) and R² is alkyl (1-8C) or wherein the proximal R¹ and R² taken together are -(CH₂)_p- wherein p = 3-5;
 R³ is H or alkyl (1-4C);
 30 R⁴ is fused or conjugated unsubstituted or substituted bicycloaryl methylene;
 n is 0, 1 or 2;
 m is 0 or 1; and

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X is OR⁵ or NHR⁵, wherein R⁵ is H or substituted or unsubstituted alkyl (1-12C), aryl (6-12C), aryl alkyl (6-16C); or

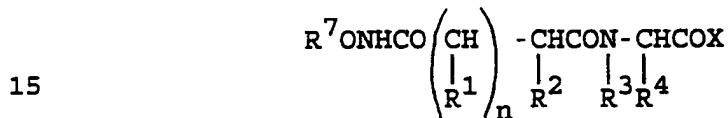
X is an amino acid residue or amide thereof; or

5 X is the residue of a cyclic amine or heterocyclic amine; and

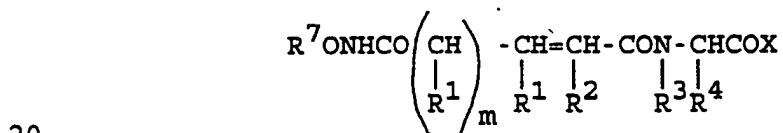
wherein R⁶ is lower alkyl (1-4C) and R⁷ is H, lower alkyl (1-4C) or acyl;

10 wherein -CONR³ is optionally in modified isosteric form;

which method comprises treating a compound of the formula



or



wherein the substituents are as above defined with an alkylating agent.

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1 / 2

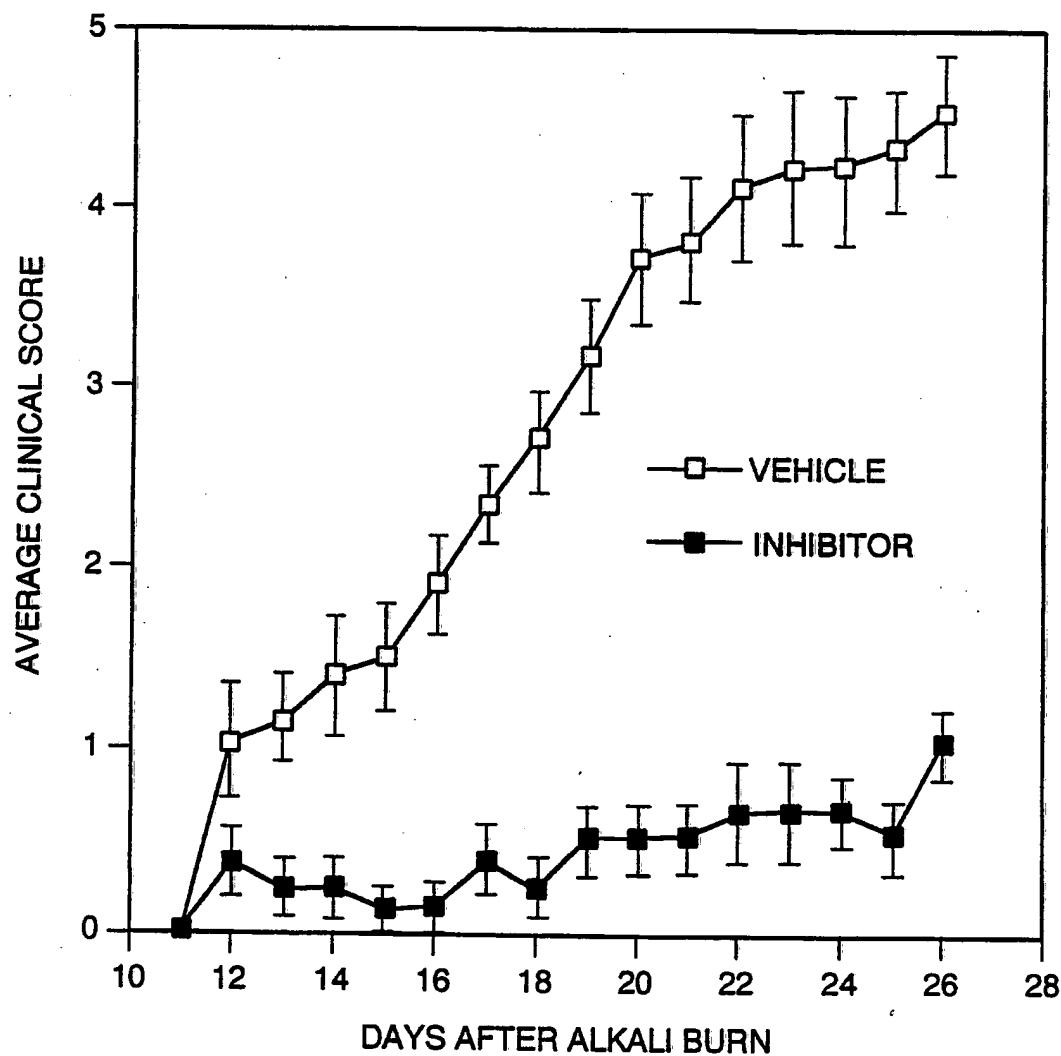
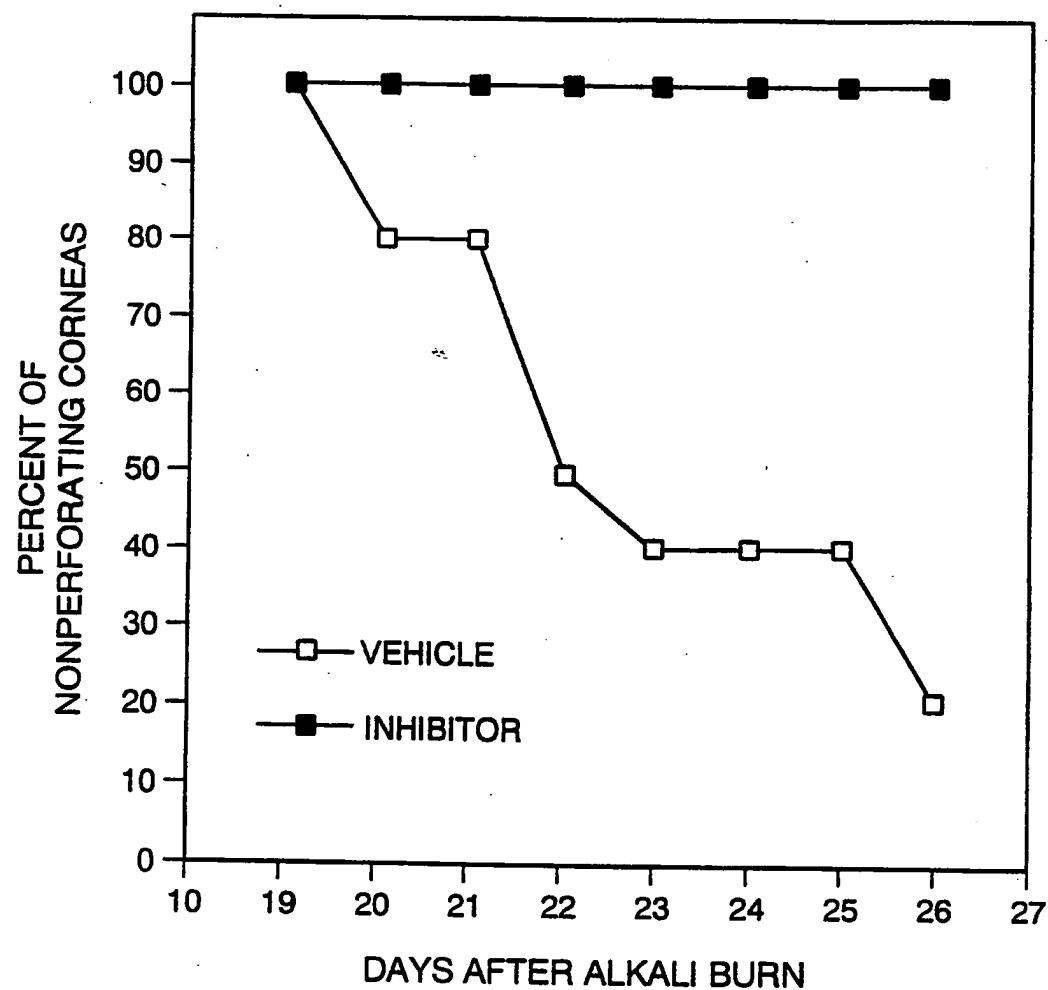


FIG. 1

SUBSTITUTE SHEET

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**FIG. 2****SUBSTITUTE SHEET**

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08723

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC
 IPC(5): C07C 290/00; A61K 31/085; C07D 209/20; A61K 31/185; A61K 31/40
 US CL : 548/495; 562/623; 560/312, 514/419; 514/575; 514/5

II. FIELDS SEARCHED

Minimum Documentation Searched ?

| Classification System | Classification Symbols |
|-----------------------|--|
| U.S. | 548/495; 562/623; 560/312; 514/575 514/419; 514/507 |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

| Category * | Citation of Document, ** with indication, where appropriate, of the relevant passages *** | Relevant to Claim No. * |
|------------|--|-------------------------|
| Y | US, A, 4,943,587 (CETENKO) 24 JULY 1990 See entire document particularly col. 5, line 51-col. 6, 1.35. | 4,6&7 |
| Y | US, A, 4,918,105 (CARTWRIGHT) 17 APRIL 1990 See entire document particularly col. 2, 1.4-col. 3, line 59. | 4,6&7 |
| Y | US, A, 4,681,894 (MURRAY) 21 JULY 1987 See entire document. | 4,6&7 |

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"C" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"D" document referring to an oral disclosure, use, exhibition or other means

"E" document published prior to the international filing date but later than the priority date claimed

"F" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"G" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"H" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"I" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

12 MARCH 1992

Date of Mailing of this International Search Report

11 MAY 1992

International Searching Authority

ISA/US

Signature of Authorized Officer

David B. Springer
David B. Springer

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers because they relate to subject matter¹³ not required to be searched by this Authority, namely:

2. Claim numbers, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

3. Claim numbers, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

As set forth in the attachment.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

indicated they would be

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 4, 6 & 7

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
 No protest accompanied the payment of additional search fees.

CONTINUATION SHEET

INVENTION I - to certain indolyl compounds of generic claims 1,2 and indolyl compounds in claim 4 with "Trp" (tryptophane group) as well as certain non-hetero containing species of claim 4, i.e., containing Ala (2-naphthal) (alamine) and pharmaceutical compositions (Cl.6) and methods of use thereof in claim 7.

INVENTIONS II-VI - drawn to compounds with certain other heterocyclic groups named in claim 2, but also contained in claim 1, as well as compositions (cl.6) and methods of use thereof in claim 7.

INVENTION II - having isoquinolinyl groups

INVENTION III - having quinolinyl groups

INVENTION IV - having pyrimidinyl groups

INVENTION V - having coumarinyl groups (and none of I-V having other heterocyclic groups as defined by R⁵ in claim 1)

INVENTION VI - having other heterocyclic groups as embraced by cyclic, amine or heterocyclic amine and compositions and methods of use thereof as in claims 6 and 7.

INVENTIONS VII-XII - to certain indolyl and other compounds with groups named by groups.

I-VI wherein "cyclic amines" are present and include at least piperidinyl as named at page 8, line 21 of the specification and compositions and methods of use thereof as in claims 6 and 7.

INVENTIONS XIII-XVIII - to certain indolyl and other compounds with groups named in group I-VI where heterocyclic amines are present and include at least morpholinyl and compositions (page 8, line 23 of specification) and methods of use thereof (claims 6 and 7).

INVENTIONS XIX-XXXV - to biochemical conjugates of the compound inventions of groups I-XVIII as embraced in claims 1-4 and specifically referred to in claim 4, as embraced by claim 5.

INVENTIONS XXXVII-LIV - to methods of inhibiting metallo protease invitro or in cell cultures (in vivo) using compounds embraced in claims 1-4, and as set forth in claim 8 embracing the compounds in Inv. I-XVIII.

CONTINUATION SHEET #2

INVENTIONS LV-LXXII - to various immuno-reactive anti-body preparations of the compounds of claims 1-4 as set forth in claim 9 embracing the compounds of I-LXXII as set forth above. Inventions LXXIII-LC to method(s) of assaying antibodies by complexes thereof with the compounds of claims 1-4, and as set forth in claim 10.

INVENTIONS XCI-XCVI - to six materially different methods of preparing the compounds of claim 1 as enumerated by claims 11-16, respectively.

The above enumerated ninety-six (96) inventions lack unity of invention since they do not form a single inventive concept in that a reference for any one of the compound compositions and method of use inventions of the groups would not render obvious any other, and searches for the various inventions are not coextensive.

The conjugate inventions of XIX-XXXVI are obviously distinct from invitro matrix and cell culture matrix metallo protease inhibition as are the immuno-reactive antibody and assaying methods inventions.

The materially different method of Groups LXXIII-XC of claims 11-16, for preparing variously the compounds of claim 1 are evidence each of these six methods is distinct from each other and each from any of the compounds of Inventions I-XXII prepared thereby.

For the above reasons, lack of unity of invention has been shown in accordance with 37 CFR 1.481(d) so that the above identified inventions are patentably distinct, have acquired a separate status in the art, and require different fields of search.

It is noted that the additional searches for the ninety-five additional inventions requires payment of \$15,200.00 (95 x \$160.00) (somewhat higher than the \$150.00/invention previously). It is noted that this Invitation was orally discussed with Ms. Kate Murashige, applicants attorney on Tuesday March 10, 1992 at the time that the non authorization of additional fees in companion case US91/08722 was made in a telecon. She also indicated that additional fees would not be paid in this case either, but herein also requested that the naphthyl containing hydroxamic acids and esters also be searched (class 562/623 and 560/312 respectively) and the corresponding 514 composition and method subclasses (514/507 and 514/575). The Examiner agreed to do so and so modified this invitation accordingly.